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INCIDENCE OF FUNGI ON YELLOW PASSION FRUIT SEEDS IN THE MARANHÃO STATE (BRAZIL)

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ABSTRACT - In recent years, passion fruit orchards have suffered from the high incidence of microorganisms, causing damage from the seedling phase to the post-harvest of the fruits; among these microorganisms are the fungi that comprise the largest number of species associated with the seeds. The objective of this work was to evaluate the incidence of fungi associated with yellow passion fruit seeds in the State of Maranhão. The experiment was carried out in 2014 and conducted at the Seed Laboratory of the State University of Southern Maranhão (UEMASUL), *Campus* Imperatriz. Fruit seeds collected from six municipalities in the state of Maranhão (Carolina, Capinzal do Norte, Estreito, Imperatriz, Vila Nova dos Martírios and São Luís) were used. A completely randomized design with four replications was adopted. A total of 400 seeds were used per municipality, 50 per gerbox, totaling the processing and analysis of 2400 seeds in general. The incidence of pathogenic agents in the seeds was determined by using the filter paper method with freezing, using four replications of 100 seeds per evaluated municipality. Seven days later, the incidence of pathogens was estimated by using a stereomicroscope, with confirmation of the genus and/or species of the fungus. The incidence of *Penicillium* sp. in yellow passion fruit seeds occurred in all assessed municipalities collected. The city of Vila Nova dos Martírios has presented the highest incidence of fungi, while the city of Imperatriz has had the lowest rate.
Keywords: *Passiflora edulis* f. *flavicarpa*, pathogens, seed health.

INCIDÊNCIA DE FUNGOS EM SEMENTES DE MARACUJÁ-AMARELO NO ESTADO DO MARANHÃO

RESUMO - Nos últimos anos, os pomares de maracujazeiro sofrem com a alta incidência de microrganismos, causando danos desde a fase de muda até a pós-colheita dos frutos, entre estes microrganismos estão os fungos que englobam o maior número de espécies associadas as sementes. Objetivou-se nesse trabalho, avaliar a incidência de fungos associados às sementes de maracujá-amarelo no Estado do Maranhão. O experimento foi realizado no ano de 2014 e conduzido no Laboratório de Sementes da Universidade Estadual do Sul do Maranhão (UEMASUL), Campus Imperatriz. Foram utilizadas sementes de frutos coletados de seis municípios do Maranhão (Carolina, Capinzal do Norte, Estreito, Imperatriz, Vila Nova dos Martírios e São Luís). Adotou-se o delineamento experimental inteiramente casualizado, com quatro repetições. Utilizou-se um total de 400 sementes por município, 50 por gerbox, totalizando o processamento e análise de 2400 sementes no total. A incidência de agentes patogênicos nas sementes foi determinada pelo método do papel filtro com congelamento, sendo utilizadas quatro repetições de 100 sementes por município avaliado. Após sete dias, a incidência dos patógenos foi estimada em microscópio estereoscópio, com confirmação do gênero e/ou espécie do fungo. A incidência do *Penicillium* sp. em sementes de maracujá-amarelo ocorreu em todos os municípios coletados. A cidade de Vila Nova dos Martírios apresentou a maior incidência de fungos, enquanto a cidade de Imperatriz ficou com o menor índice.
Palavras-chave: *Passiflora edulis* f. *flavicarpa*, patógenos, sanidade de sementes.

INTRODUCTION

The passion fruit tree is cultivated in tropical and subtropical climates. It belongs to the *Passifloraceae* family and the *Passiflora* genus (COELHO et al., 2016). There are more than 150 species of passion fruit (SANTOS et al., 2017). However, the most common species in Brazil and the world are the yellow passion fruit (*Passiflora edulis f. flavicarpa*), purple passion fruit (*Passiflora edulis*), and fragrant gradanilla (*Passiflora alata*) (RIBEIRO et al., 2018). Nevertheless, the yellow or sour passion fruit represents almost all of the fruit volume commercialized in the world (KISHORE et al., 2011).

Nowadays, Brazil is the major producer and consumer of yellow passion fruit in the world. Brazilian production was around 1 million tons (IBGE, 2020), and almost its entirety was destined for the internal market. The regions with larger production are the South,

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Southeast, and Northeast, especially Bahia, which has the largest production in the country (FREITAS et al., 2020).

According to Faleiro et al. (2015), the multiple and diversified use of passion fruit involve the consumption of raw fruit (sweet passion fruit), juices (sour passion fruit), and flowers (ornamental passion fruit). Several species of the *Passiflora* genus present functional medical properties used by cosmetic, pharmaceutical, and nutritional industries (ZERAIK et al., 2010).

Seeds, cuttings, and grafting may propagate the passion fruit tree. Nevertheless, by being cheaper and having easier execution, propagation by seeds is the most common method, as seedlings propagated by seeds are a viable alternative, requiring easier conservation and storage. Moreover, the main cultivars available in the market use the propagation by seeds (FALEIRO; JUNQUEIRA, 2016). A disadvantage is that the seed is the most efficient vector for disseminating pathogens (fungi and bacteria) due to its intrinsic properties. As the pathogen originated by the seed has more chances of causing diseases in the plant, it can then be spread to other healthy plants, starting an epidemy (PARISI et al., 2018).

The cultivation of sour passion fruit is of great economic and social importance in Brazil. Still, there is a lack of information about the pathology of its seeds. However, it is known that damages caused by diseases transmitted by seeds occur mainly during the stages of germination and formation of seedlings. Thus, this paper aims to evaluate the incidence of fungi associated with seeds of yellow passion fruit.

MATERIAL AND METHODS

The experiment was carried out in 2014 and conducted at the Laboratory of Seeds of the Universidade Estadual do Sul do Maranhão (UEMASUL), Imperatriz *Campus*. Seeds from fruits collected in six municipalities of the state of Maranhão [Carolina (7°20'16" S, 47°28'4" W), Capinzal do Norte (4°43'42" S, 44°19'37" W), Estreito (5°46'60"S, 43°15'0" W), Imperatriz (5°31'32" S, 47°28'37" W), Vila Nova dos Martírios (5°11'16" S, 48°8'9" W), and São Luís (2°31'51" S, 44°18'24" W)] were used. To compose the treatments, the seeds were divided according to their respective municipality. In each municipality, fruit samples were collected from different farms. The samples were collected in the morning, being them ten ripe fruits of different sour passion fruit trees, randomly selected by walking in "W" in each orchard sampled. The samples were collected between the months of September-November 2014, with pruning shears duly disinfected and aseptically stored in plastic bags, which were immediately taken to the Laboratory of Seeds.

In the laboratory, the fruits were transversally cut and submitted to mucilage mechanical remotion by attrition of pulp in a sieve with a mesh of 1 mm and sterilized textile towels. After that, the seeds were dried on paper towels for a period of 48 h.

For sanitary analysis, transparent acrylic boxes of Gearbox models (11 x 11 x 3,5 cm) were used, being them previously sterilized with 70% alcohol with three blotter paper sheets previously sterilized (125ºC for 30 min) in its bottom, humidified with distilled water with 2,5 times its weight. Hence, the seeds were stored uniformly on the papers and placed at the B.O.D. chamber with a constant temperature of 20±2ºC, relative air humidity of 80-85%, and a photoperiod of 12 h.

Using four repetitions of 100 seeds by sample (municipality), the freeze filter paper method was used to determine the pathogenic agent incidence in seeds. After seven days, at 20ºC and 12 h of light, the incidence of pathogens was estimated in a binocular stereomicroscope (Opton Tim 30). The fungi genus and species were confirmed following Barnet and Hunter (1986), and semi-permanent slides were mounted for all the samples of seeds with incidence.

A completely randomized design was adopted, containing eight repetitions and 50 seeds by repetition. A total of 400 seeds by sample (municipality) and 50 seeds by gearbox were used; thus, 2,400 seeds were processed and analyzed. The results obtained were submitted to variance analysis, and the means were compared by the Tukey test at a 5% error probability, using the statistical software Sisvar (FERREIRA, 2011).

RESULTS AND DISCUSSION

In the analysis of yellow passion fruit seeds were detected 11 genera of fungi (Table 1) in different municipalities of Maranhão. The incidence of *Penicillium* sp. was verified in all samples. The presence of *Drechslera* sp. and *Epicoccum* sp. was verified only in the fruits collected in Carolina and *Aspergillus candidus* in the seeds of fruits collected in Estreito. The fungi that presented higher incidence were those of the genera *Penicillium* sp. and *Aspergillus* sp, which developed fast, reducing the seeds' viability (NASCIMENTO et al., 2006). They are associated with the deterioration of seeds at inadequate storage conditions, and their contamination can occur soon after the harvest.

Similar results were found by Santana et al. (2018) while evaluating the incidence of diseases of fungal etiology in bitter passion fruit (plants and fruits) in the municipality of Alta Floresta (state of Mato Grosso). This study identified fungi of the genera *Colletotrichum* sp., *Fusarium* sp., *Rhizoctonia* sp., *Aspergillus* sp., *Curvulan'a* sp., *Geotrichum* sp., *Helminthosporium* sp., *Phytomice* sp., *Phoma* sp. and *Penicillium* sp. Parisi et al. (2018), obtaining information about the transmission and pathogenicity of fungi by sanitary test in seeds of sweet passion fruit, verified in their collection the occurrence of fungi of the genera *Alternaria* sp. (4%), *Botrytis* sp. (12%), *Cladosporium* sp. (16%), *Fusarium* sp. (9%) e *Lasiodiploidia* sp. (4%). After isolation and inoculation of the seeds, the incidence of these fungi increased 100%. Cerqueira et al. (2019), evaluating the physiological quality of bitter passion fruit seed in the state of Bahia, identified the fungi *Fusarium* sp. (50,75%), *Lasiodiplodia* sp. (14,71%), *Penicillium* sp. (18,32%), *Colletotrichum* sp. (6,61%), *Cladosporium* sp. (3,30%), *Aspergillus* sp.

(4,80%), *Alternaria* sp. (0,60%) and *Trichoderma* sp. (0,90%).

TABLE 1 - Incidence (%) of fungi in seeds of yellow passion fruits from seven municipalities of the state of Maranhão.

	Municipalities of Maranhão					
Pathogens	Carolina	Estreito	Imperatriz	Vila Nova dos Martírios	São Luís	Capinzal do Norte
Alternaria sp.	-	0.50	-	-	9.00	-
Aspergillus candidus	-	4.00	-	-	-	-
Aspergillus níger	-	54.50	0.50	16.00	1.75	1.50
Aspergillus flavus	-	-	-	14.00	1.25	4.75
Cladosporium sp.	0.50	1.50	0.25	-	-	
Colletotrichum sp.	0.25	-	0.50	-	-	0.75
Drechslera sp.	1.00	-	-	-	-	-
Epicoccum sp.	0.50	-	-	-	-	-
Fusarium oxysporum	-	-	-	1.50	3.25	3.00
Penicillium sp.	0.50	10.25	0.50	81.25	0.25	6.75
Phoma sp.	-	1.00	-	-	-	0.25
Rhizopus stolonifer	13.5	6.25	-	20.00	0.75	0.50
Trichoderma sp.	0.75	2.25	0.25	-	-	6.00

For the mean data of incidence (%) of fungi in seeds of yellow passion fruit (Table 2), it is observed that those collected in the municipality of Vila Nova dos Martírios presented higher incidence (65,63%) differing statistically from the samples collected in the other municipalities. The incidence of fungi in the seeds of the fruits collected was observed at 11,88% in Capinzal do Norte, 9,13% in Carolina, and 8,13% in São Luís, not presenting statistical differences between them. The seeds of the fruits collected in Imperatriz presented a lesser incidence of fungi (1%). These seeds' lesser fungal incidence is probably due to using fungicides (Captan and Tiram) by producers in the area. Control of diseases in passion fruit trees has been performed basically by the aerial application of fungicides and bactericides, which has not always produced satisfying results, as the pathogens present resistance to the active ingredients (PERUCH et al., 2018; NUNES et al., 2018).

TABLE 2 - Mean data of incidence (%) of fungi in yellow passion fruit seeds from seven municipalities of the state of Maranhão.

Municipalities of Maranhão	Averages
Vila Nova dos Martírios	65.63 a*
Estreito	40.13 b
Capinzal do Norte	11.88 c
Carolina	9.13 c
São Luís	8.13 c
Imperatriz	1.00 d
CV (%)	20.07

*Means followed by equal letters do not differ among them by the Tukey test at a 5% probability of error.

Lazarotto et al. (2013) presented that the fungicide Captan's use reduced the average incidence of *Colletotrichum* sp., *Fusarium* sp., *Penicillium* sp., *Phomopsis* sp., *Rhizoctonia* sp. in cedar (*Cedrela fissilis)* seeds. A study developed by Silva et al. (2011), using Captan and Tiram, presented a reduction of *Fusarium* sp., *Penicillium* sp., *Alternaria* sp. e *Aspergillus* sp. in seeds of forest species.

The seeds from fruits collected in cities more distant from the analysis site presented a higher infestation of the genera *Penicillium* and *Aspergillus* storage fungi. Factors such as temperature and humidity contributed to such results since the storage of fruits for transportation was in plastic bags. The closed bags became an ideal environment (hot and humid) for the development of such microorganisms.

Trigiano et al. (2011) consider that temperature and humidity are the more important factors that interfere with the disease cycle. Temperature is an essential factor in the growth of pathogens. The severity of a disease can be higher in areas, seasons, or years with colder temperatures, while other pathogens develop better in high temperatures (AMORIM et al., 2018). According to Jaronski (2010), fungi require high humidity to start their development.

For the good management of the crops, the knowledge of the pathogens that may affect an orchard is necessary to control them. Laboratory tests to identify fungi in yellow passion fruit seeds seem to be good practices with easy execution and reliable results when well conducted. When fungi are identified, it is possible to map viable areas to implement crops and increase producers' awareness about their farms' management.

CONCLUSIONS

In samples of all municipalities studied, *Penicillium* sp. occurred in yellow passion fruit's seeds.

The city of Vila Nova dos Martírios presented a higher incidence of fungi, while the city of Imperatriz presented a lower incidence.

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